Figure 1. Baseline clinical parameters (mean ± SD) in both mild to moderate (NYHA I-II) and severe (NYHA III-IV) congestive heart failure (CHF) groups.

	Mild to Moderate CHF n = 40	Severe CHF n = 47
Age (years)	62 ± 7	67 ± 8 §
Ejection fraction (%)	29 ± 4	20 ± 6 §§
Gender (Male/Female)	36/4	38/9
Ischemic (n)	38	38
Non-ischemic (n)	2	9

§ p<0.01, §§ p<0.001 severe vs. mild to moderate CHF

Figure 2.
Kaplan-Meier survival cuves in patients with mild to moderate (NYHA Class I-II) and severe (NYHA Class III-IV) congestive heart failure (CHF)
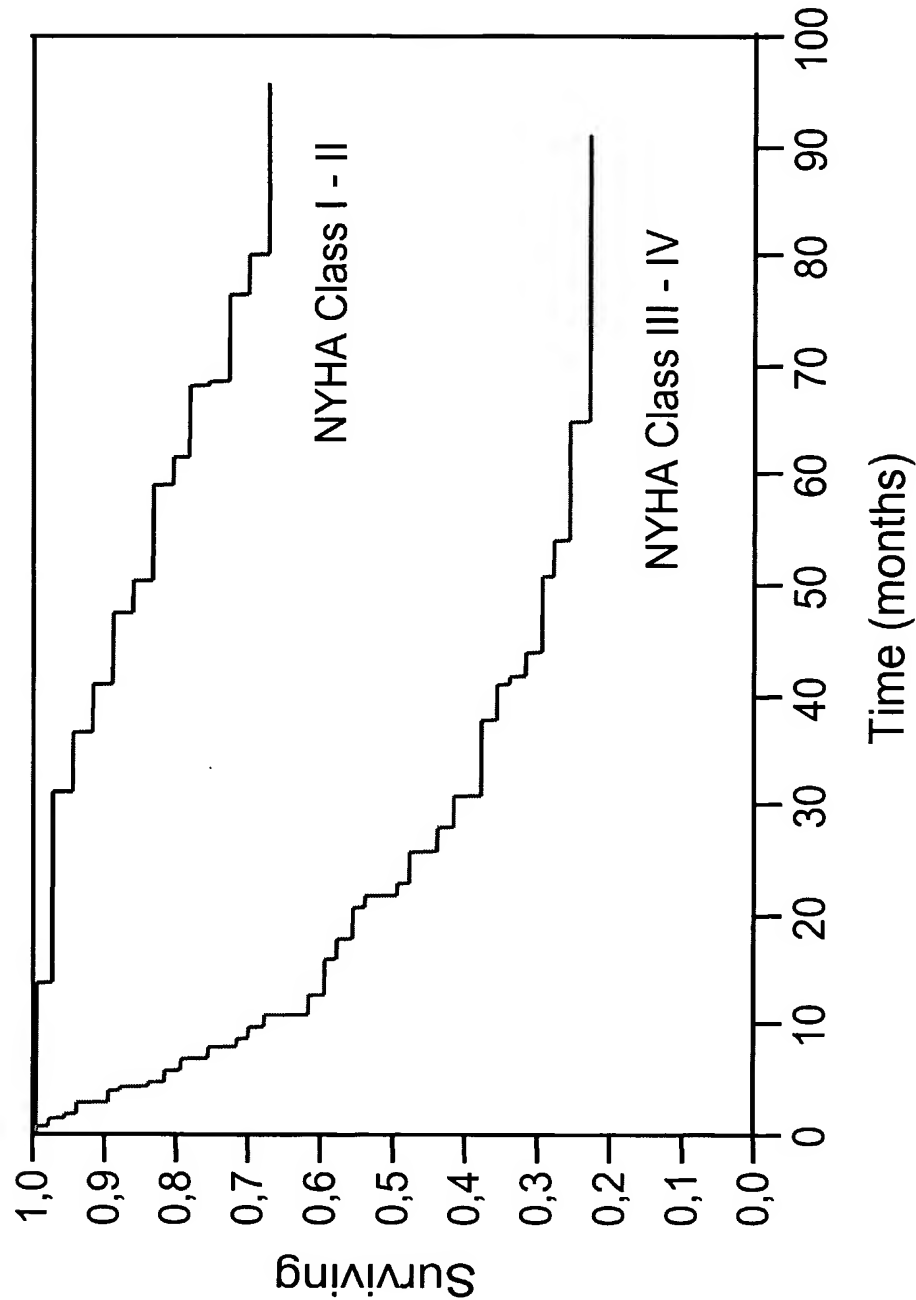
Figure 3. Values for the natriuretic and vasoactive peptides in healthy control individuals

Parameter	Method	Specificity	Units	n	Geometrical mean	99.5 % Confidence Limits of the mean	
						Low limit	High limit
Big-ET-1 (1-38)	ELISA (2-site)	C-proET-1 (1-38)	fmol/ml	50	0.96	0.81	1.16
Big-ET-1 (22-38)	RIA	C-proET-1 (1–38) and fragments of Big-ET-1 with epitopes between (22-38)	pg/ml	92	5.5	5.3	5.8
ET-1	RIA	ET-1 (1 – 21) and peptides with epitopes between (1-21)	pg/ml	92	4.1	3.8	4.4
N-proANP (1-98)	ELISA (2-site)	N-proANP (1-98)	fmol/ml	60	1692	1466	1952
N-proANP (1-25)	RIA	N-proANP (1 – 98) and peptides with epitopes between (1-25)	pg/ml	92	167	149	188
N-proANP (68-98)	RIA	N-proANP (1 – 98) and peptides with epitopes between (68-98)	pg/ml	92	268	236	303
BNP	IRMA (2-site)	BNP (77-108)	pg/ml	28	9	5	15
BNP	RIA	BNP (77 – 108) and peptides with epitopes between 77-108 of N-proBNP	pg/ml	92	5.0	4.4	5.6
N-proBNP	ELISA (Competition)	BNP (1 – 76) and peptides with epitopes between (1-76)	fmol/ml	50	267	224	319

Figure 4. Baseline neurohormonal data [geometric mean (range)] in

both mild to moderate and severe CHF groups,

and comparison with healthy controls.

	Controls [φφ]	Mild to Moderate CHF n = 40	Severe CHF n = 47
N-proANP (1-98) fmol/ml	1692 (212-3908)	3192 (1001-7768)*	5528 (1129-18180)**§§
N-proANP (1-25) pg/ml	167 (66-354)	537 (145-2010)	1903 (623-7148)**§§
N-proANP (68-98) pg/ml	268 (94-898)	860 (164-2480)*	2507 (709-9075)**§§
N-proBNP fmol/ml	267 (95-1097)	491 (109-1610)	1521 (598-5491)**§§
BNP IRMA pg/ml	9 (2-49)	43 (3-212)	328 (28-1610)**§
BNP RIA pg/ml	5 (3-15)	22 (10-64)*	75 (15-192)**§§
ET-1 pg/ml	4.1 (2.1-6.6)	3.5 (0.9-8.3)	10.0 (8.7-11.6)**§§
BIG ET-1 RIA pg/ml	5.5 (4.0-7.6)	7.2 (3.8-12.2)	12.0 (5.8-22.9)**§§
BIG ET-1 ELISA fmol/ml	1.0 (0.4-2.8)	1.8 (0.6-4.5)	3.8 (1.3-14.5)**§§

*p<0.05, ** p<0.001 vs controls
§ p<0.01, §§ p<0.001 severe vs mild to moderate CHF
[φφ] Data derived from Figure 3

Figure 5. Prognostic values for survival using natriuretic and vasoactive peptide assays, considered independently in NYHA III-IV patients *

Prognostic parameters considered independently			Best estimate of cutoff level segregating the patients with good and poor survival prognosis			Median survival time estimation (months)		Test between groups Prob>ChiSq Wilcoxon
			Value of the cutoff level	Cutoff levels expressed as fold geometric mean of normal	Cutoff levels expressed as fold 99.5% Confidence Limits of the geometrical mean	Bad prognosis group	Good Prognosis group	
Big-ET-1 (1-38)	2-site ELISA	fmol/ml	4.0	4.1	3.5	5.0	10	31	0.0005
Big-ET-1 (22-38)	RIA	pg/ml	13.8	2.5	2.4	2.6	10	30	0.0255
ET-1	In-house RIA	pg/ml	8.4	2.1	1.9	2.2	9	30	0.0149
N-proANP (1-98)	2-site ELISA	fmol/ml	6483	3.8	3.3	4.4	10	43	0.003
N-proANP (1-25)	In-house RIA	pg/ml	1784	10.7	9.5	12.0	13	44	0.0204
N-proANP (68-98)	In-house RIA	pg/ml	2562	9.6	8.5	10.8	11	31	0.0092
BNP	IRMA	pg/ml	480	54	32	89	10	28	0.1238
BNP	In-house RIA	pg/ml	92	18	16	21	9	28	0.0577
N-proBNP	Competition-ELISA	fmol/ml	1512	5.7	4.7	6.8	11	42	0.0423

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

Figure 6. Patients with severe CHF (NYHA Class III-IV)
A : Big ET-1 (1-38) <4.0 pg/ml
B : Big ET-1 (1-38) >4.0 pg/ml
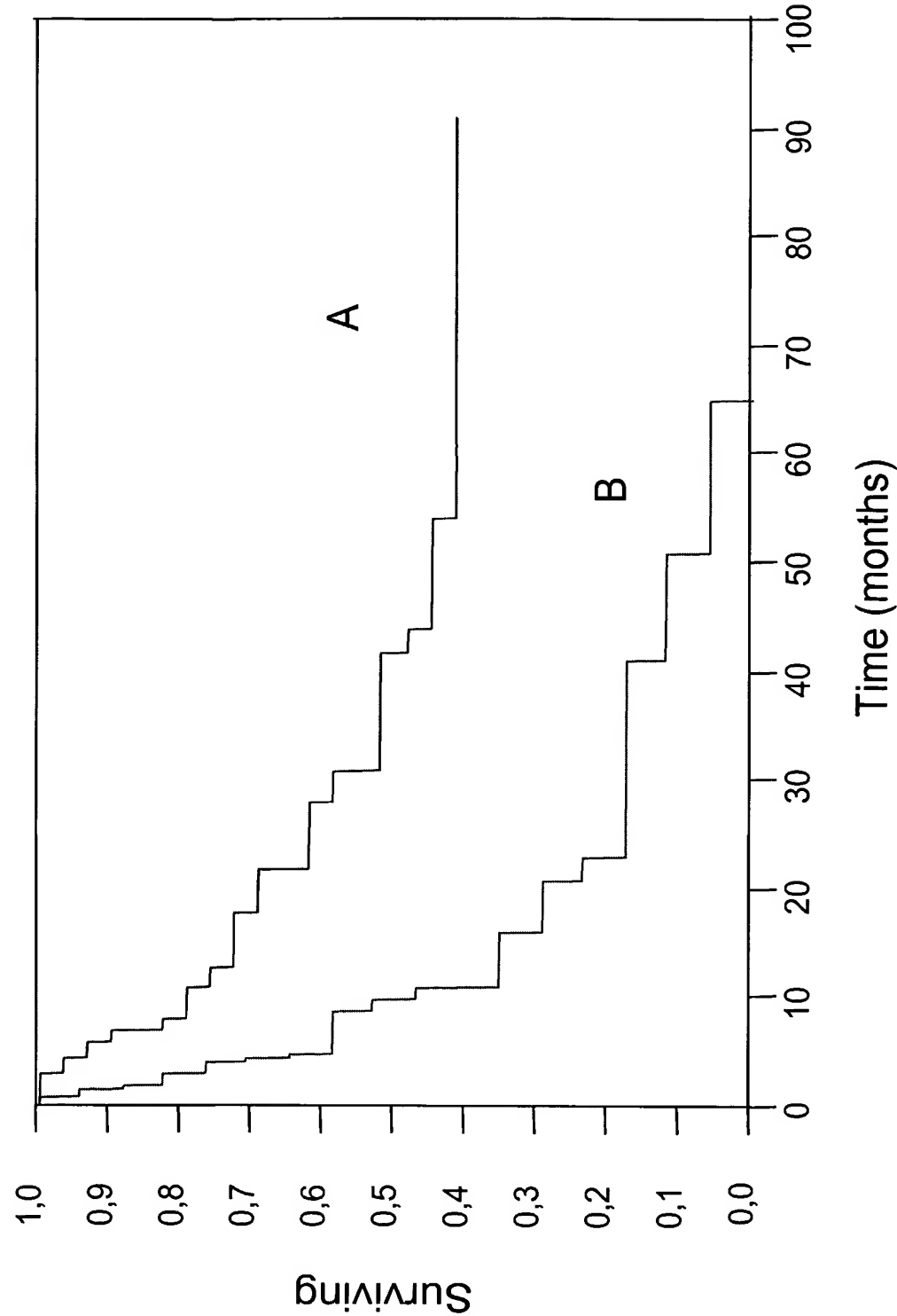
Figure 7. Patients with severe CHF (NYHA Class III-IV)
A : N-pro ANP (1-98) <6483 fmol/ml
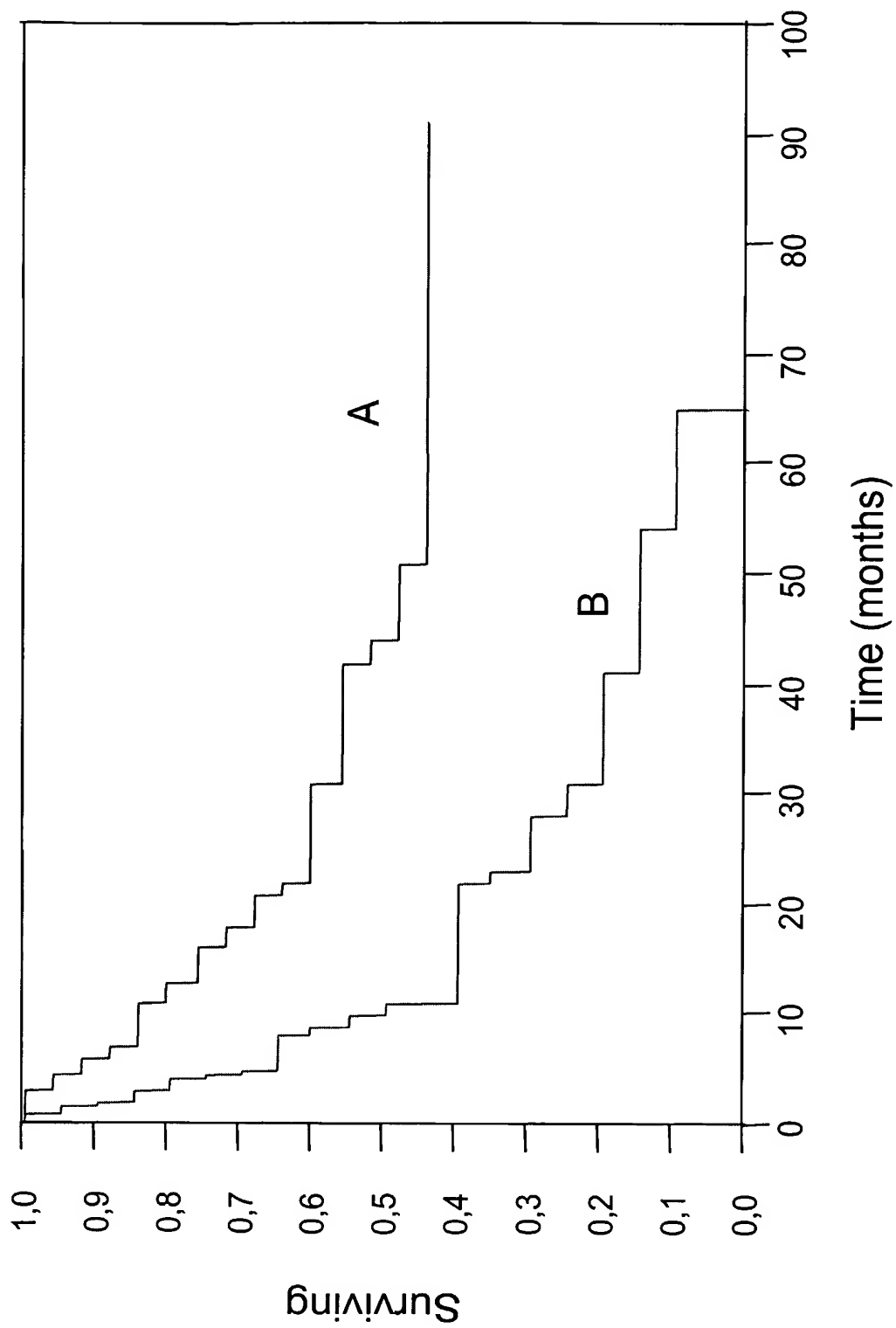B : N-pro ANP (1-98) >6483 fmol/ml

Figure 8. Patients with severe CHF (NYHA Class III-IV)
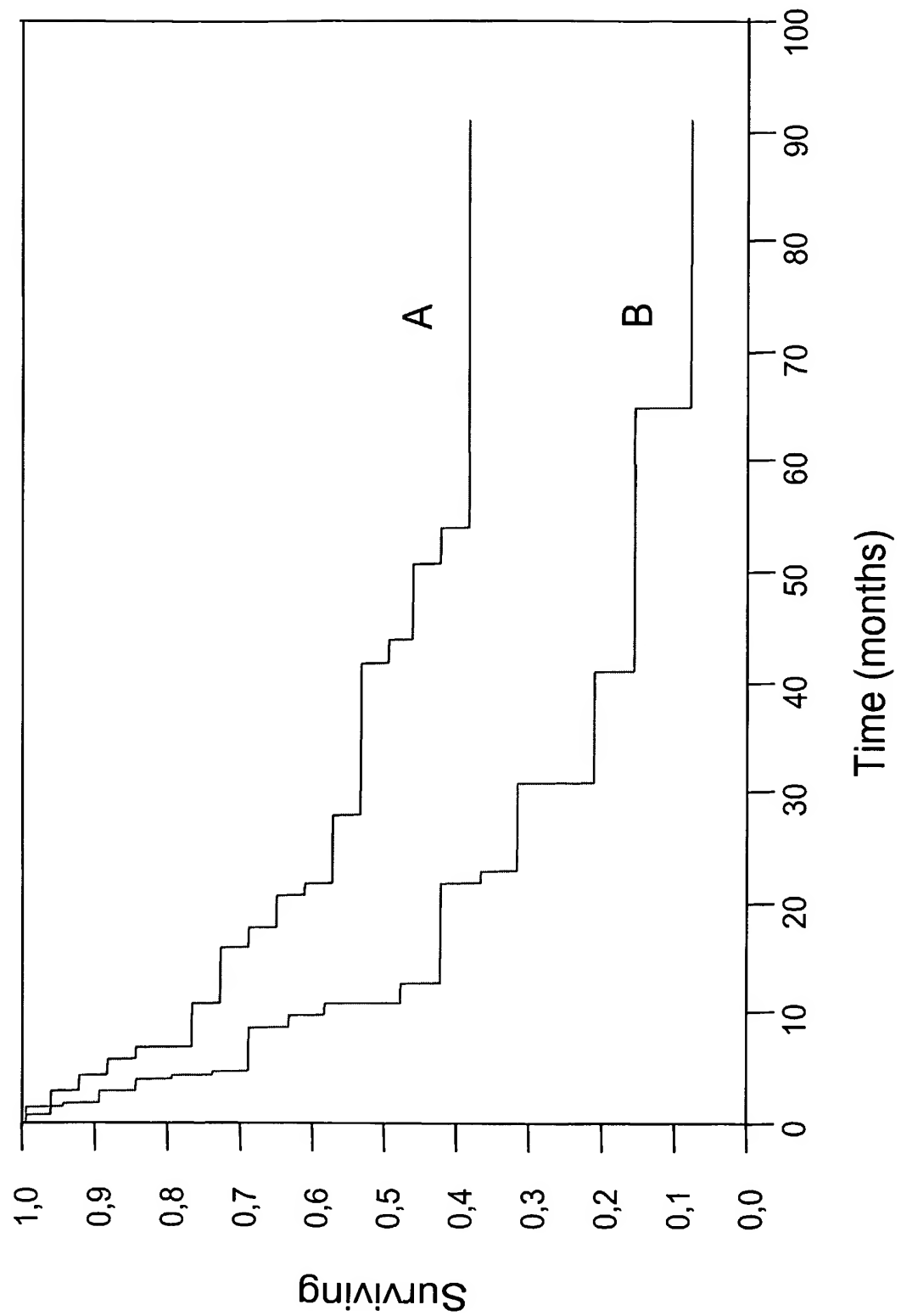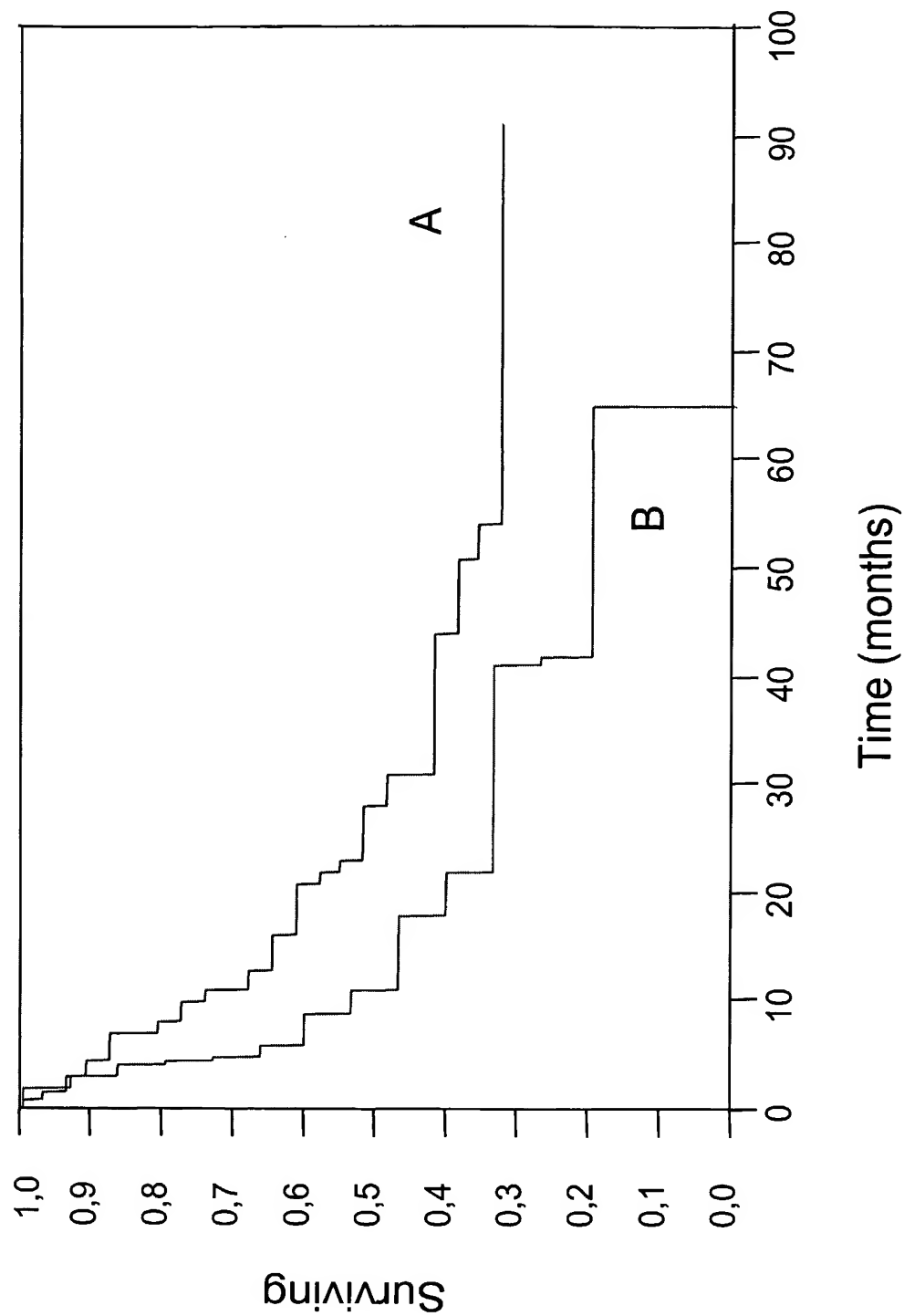A : N-proBNP <1512 fmol/ml
B : N-proBNP >1512 fmol/ml

Figure 9. Patients with severe CHF (NYHA Class III-IV)
A : BNP (IRMA) <480 pg/ml
B : BNP (IRMA) >480 pg/ml

Figure 10. Prognostic values for survival using Big-ET-1 (1-38) testing determined with a specific 2 site ELISA, used in combination with several other natriuretic and vasoactive peptide assays in NYHA III-IV patients *

Parameters considered in combination		Classification based on cut-off combinations (Cutoff levels are as in Figure 5) L : marker below cut-off H : marker above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)							
Big ET-1 (1-38) (2-site ELISA)	N-proANP (2-site ELISA)	M1 L and M2 L	> 91	10	11	21	45	0.0002
		M1 H and M2 L OR M1 L and M2 H	22	10	1	11		
		M1 H and M2 H	5	13	0	13		
Big ET-1 (1-38) (2-site ELISA)	N-proANP (1-25) (RIA)	M1 L and M2 L	60	11	11	22	46	0.0013
		M1 H and M2 L OR M1 L and M2 H	16	11	1	12		
		M1 H and M2 H	5	12	0	12		
Big ET-1 (1-38) (2-site ELISA)	N-proANP (68-98) (RIA)	M1 L and M2 L	>91	8	10	18	46	0.0003
		M1 H and M2 L OR M1 L and M2 H	21	14	2	16		
		M1 H and M2 H	4.5	12	0	12		

Parameters considered in combination		Classification based on cut-off combinations (Cutoff levels are as in Figure 5) L : marker below cut-off H : marker above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)							
Big ET-1 (1-38) (2-site ELISA)	N-proBNP Competition one site ELISA	M1 L and M2 L	61	9	9	18	41	0.0009
		M1 H and M2 L OR M1 L and M2 H	22	8	2	10		
		M1 H and M2 H	7.5	13	0	13		
Big ET-1 (1-38) (2-site ELISA)	BNP (IRMA)	M1 L and M2 L	44	12	10	22	46	0.0044
		M1 H and M2 L OR M1 L and M2 H	16	14	2	16		
		M1 H and M2 H	4.5	8	0	8		
Big ET-1 (1-38) (2-site ELISA)	BNP (RIA)	M1 L and M2 L	31	13	8	21	46	0.0011
		M1 H and M2 L OR M1 L and M2 H	20	11	4	15		
		M1 H and M2 H	4.5	10	0	10		

Parameters considered in combination		Classification based on cut-off combinations (Cutoff levels are as in Figure 5) L : marker below cut-off H : marker above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)							
Big ET-1 (1-38) (2-site ELISA)	ET-1 (RIA)	M1 L and M2 L	37	10	7	17	36	0.0055
		M1 H and M2 L OR M1 L and M2 H	12	10	1	11		
		M1 H and M2 H	4.5	8	0	8		

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

Figure 11. Patients with severe CHF (NYHA Class III-IV)
A : Big-ET-1 (1-38) <4.0 pg/ml AND N-proANP (1-98) <6483 fmol/ml
B : Big-ET-1 (1-38) >4.0 pg/ml OR N-proANP (1-98) >6483 fmol/ml
C : Big-ET-1 (1-38) >4.0 pg/ml AND N-proANP (1-98) >6483 fmol/ml
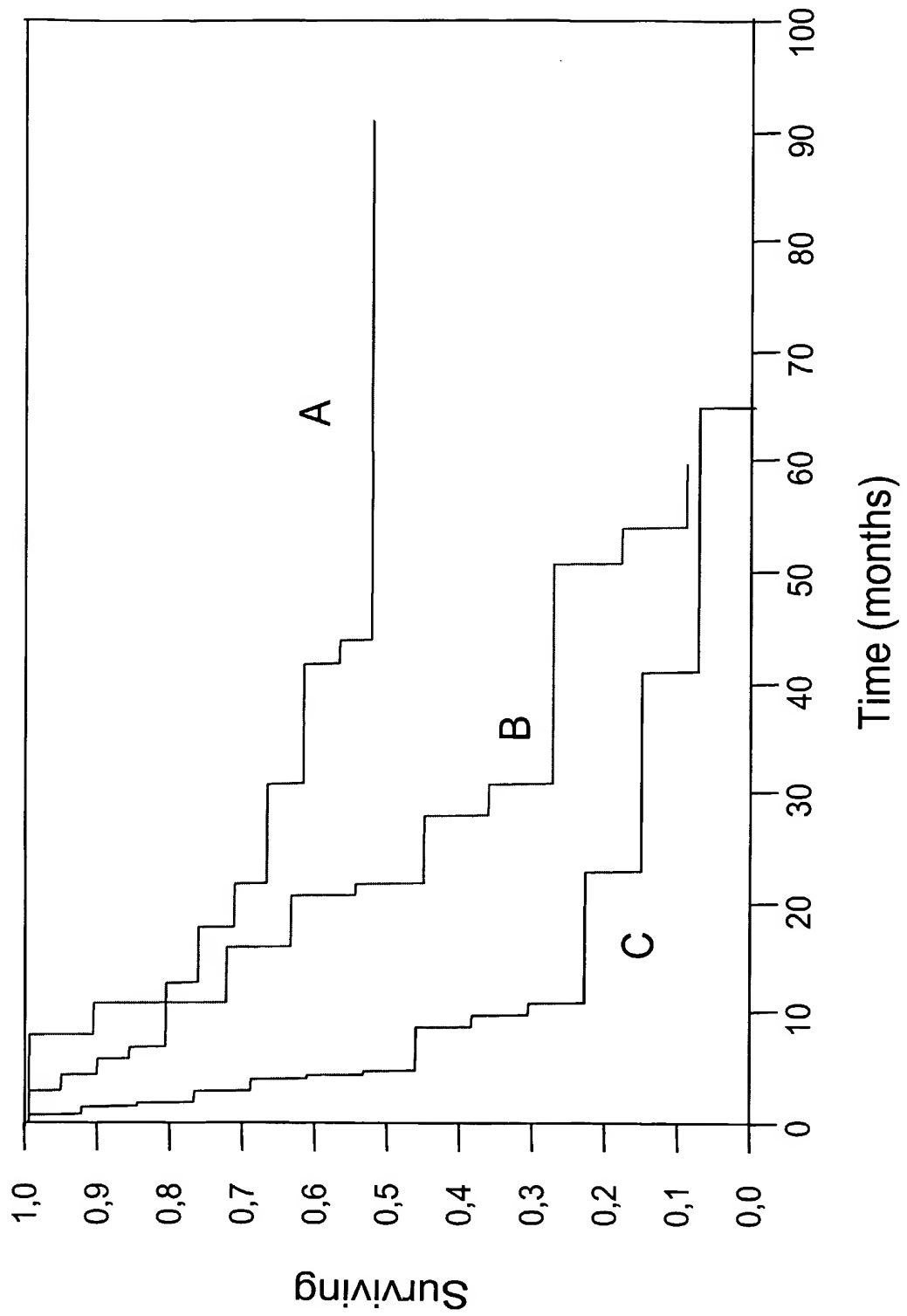
Figure 12. Patients with severe CHF (NYHA Class III-IV)
A : Big ET-1 (1-38) <4.0 pg/ml AND N-proANP (1-98) <6483 fmol/ml
B : Big ET-1 (1-38) >4.0 pg/ml AND N-proANP (1-98) >6483 fmol/ml
Solid lines: survival
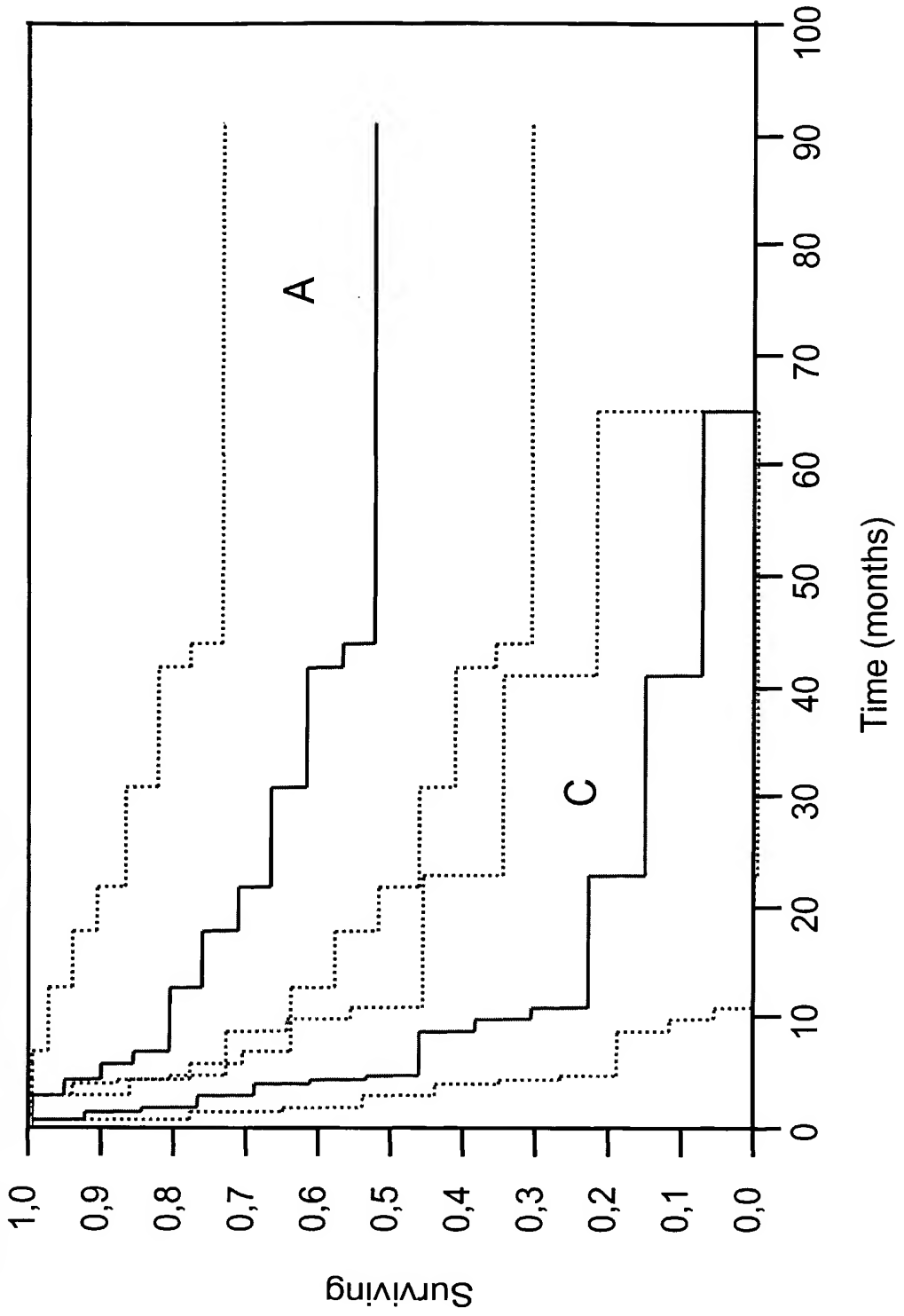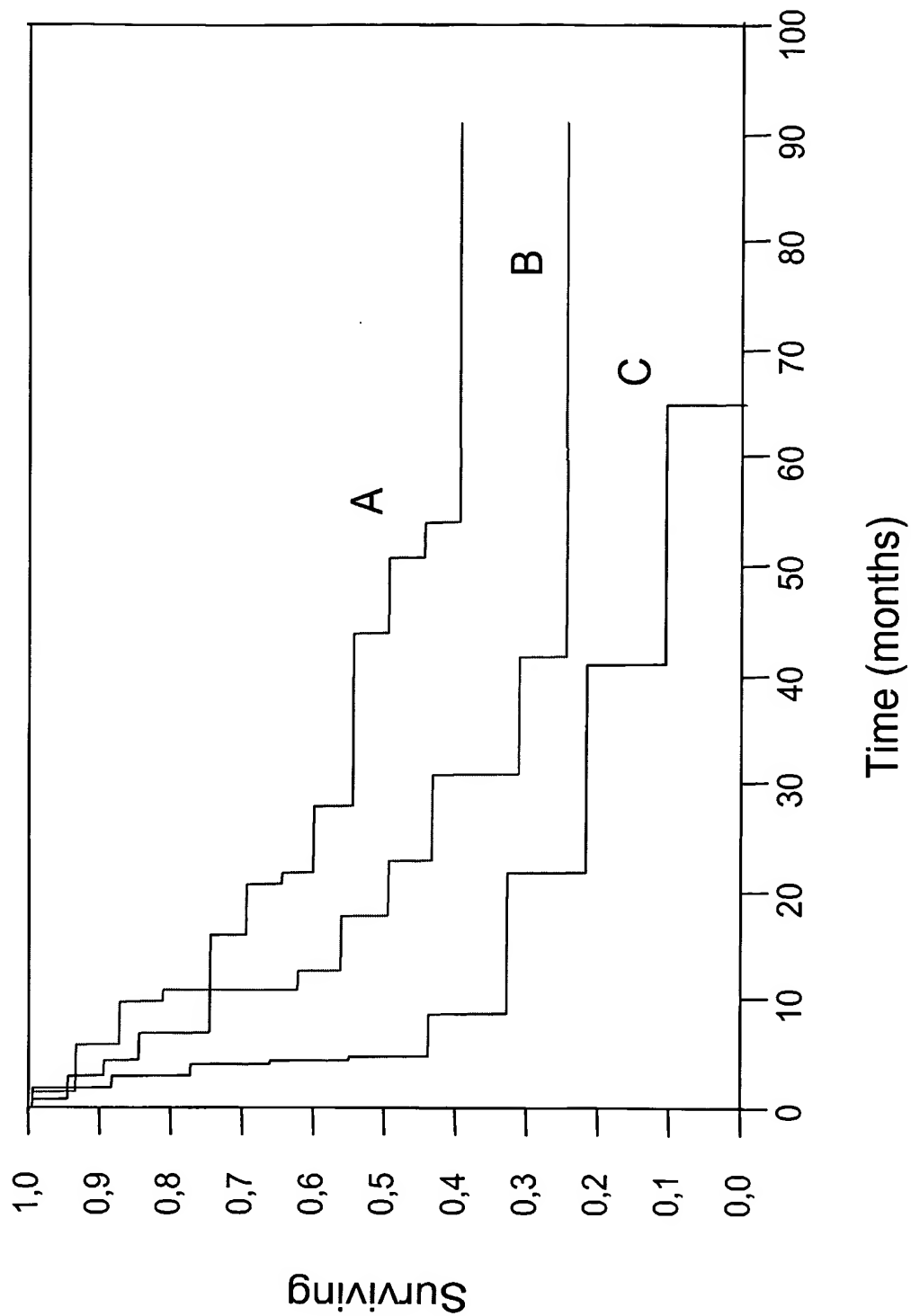Dotted lines: confidence limits

Figure 13. Prognostic values for survival using N-proBNP testing determined with a single site competition ELISA used in combination with 2 different BNP assays in NYHA III-IV patients *

Parameters considered in combination		Classification based on cut-off combinations (Cut-off levels are as in Fig. 5) L : below cut-off H : above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1	Marker 2							
N-proBNP Competition one site ELISA	BNP IRMA	M1 L and M2 L	44	12	8	20		
		M1 H and M2 L OR M1 L and M2 H	18	12	4	16	45	0.0282
		M1 H and M2 H	5	9	0	9		
N-proBNP Competition one site ELISA	BNP RIA	M1 L and M2 L	44	11	8	19		
		M1 H and M2 L OR M1 L and M2 H	18	13	3	16	45	0.0343
		M1 H and M2 H	5	9	1	10		

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

Figure 14. Patients with severe CHF (NYHA Class III-IV)
A : N-proBNP <1512 fmol/ml AND BNP (IRMA) <480 pg/ml
B : N-proBNP >1512 fmol/ml OR BNP (IRMA) >480 pg/ml
C: N-proBNP >1512 fmol/ml AND BNP (IRMA) >480 pg/ml

Figure 15. Patients with severe CHF (NYHA Class III - IV)
A : N-proBNP <1512 fmol/ml AND BNP (IRMA) <480 pg/ml
C: N-proBNP >1512 fmol/ml AND BNP (IRMA) >480 pg/ml
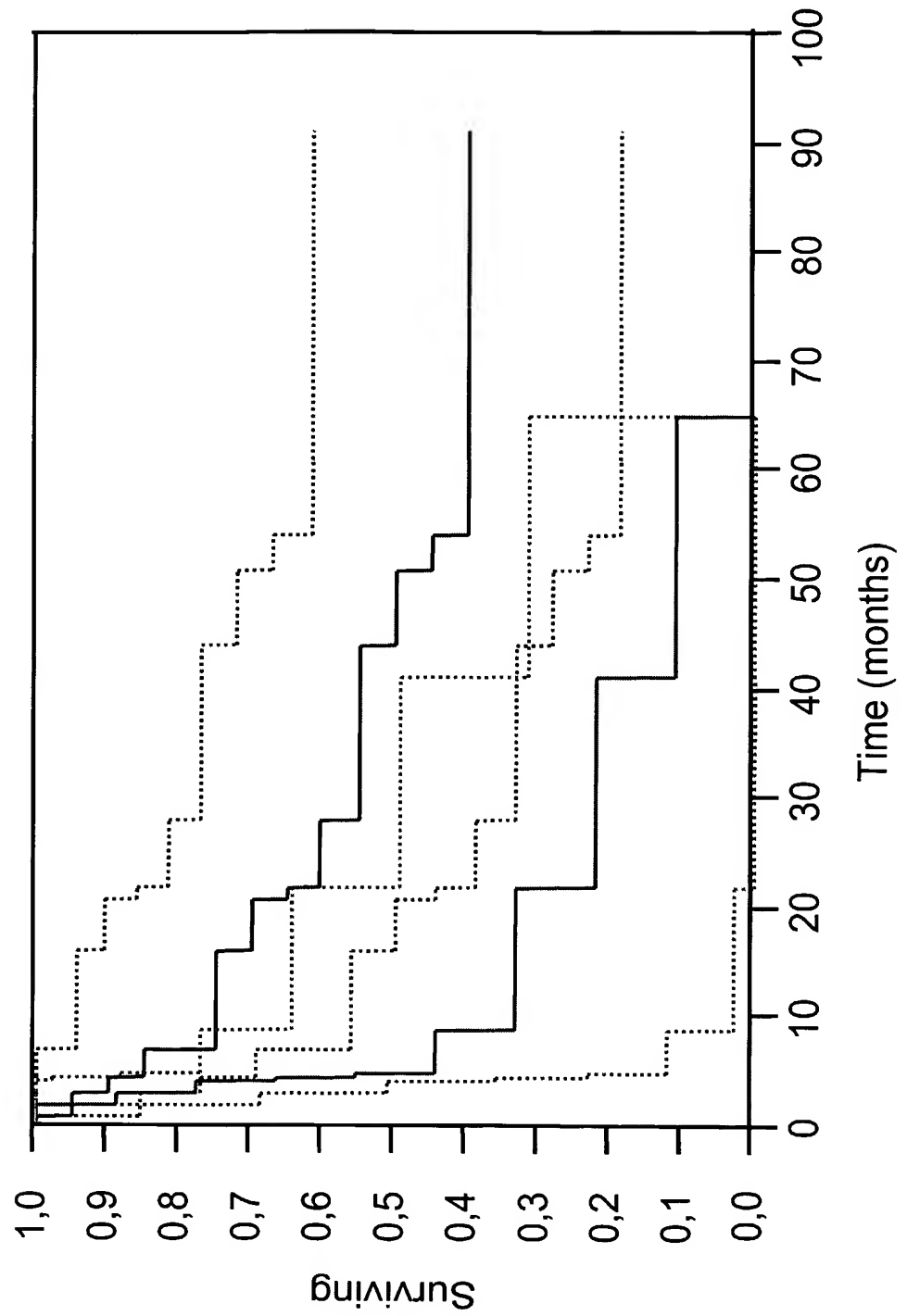Solid lines: survival
Doted lines: confidence limits

Figure 16. Prognostic values for survival using several natriuretic and vasoactive peptide assays, considered independently in NYHA I - II patients *

Prognostic parameters considered independently			Value of the cutoff level	Cutoff levels expressed as fold geometric mean of normal	Cutoff levels expressed as fold 99.5% Confidence Limits of the geometrical mean	Classification based on cut-off L.: below cut-off H: above cut-off	75% survival estimation (months)	% Survival at end of study	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Test between groups Prob>ChiSq Wilcoxon
N-proANP (1-98)	2-site ELISA	fmol/ml	3292	1.9	1.7 2.2	L	> 91	84	3	16	19	0.0107
						H	43	47	9	8	17	
N-proANP (1-25)	In-house RIA	pg/ml	496	3.0	2.6 3.3	L	> 91	79	4	15	19	0.0772
						H	49	55	8	10	18	
N-proANP (68-98)	In-house RIA	pg/ml	736	2.8	2.4 3.1	L	>91	87	2	13	15	0.0474
						H	60	54	10	12	22	
BNP	IRMA	pg/ml	38	4.2	2.6 7.0	L	>91	81	3	13	16	0.1078
						H	50	57	9	12	21	
BNP	In-house RIA	pg/ml	25	5.0	4.4 5.7	L	>91	81	4	18	22	0.0178
						H	47	57	8	7	15	

| Prognostic parameters considered independently			Best estimate of cutoff level segregating patients with good and poor survival prognosis			Classification based on cut-off L : below cut-off H : above cut-off	75% survival estimation (months)	% Survival at end of study	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Test between groups Prob>ChiSq Wilcoxon
			Value of the cutoff level	Cutoff levels expressed as fold geometric mean of normal	Cutoff levels expressed as fold 99.5% Confidence Limits of the geometrical mean							
N-proBNP	Competition-ELISA	fmol/ml	404	1.5	1.3 1.8	L	>91	86	2	12	14	0.0755
						H	53	56	10	13	23	
Big-ET-1 (1-38)	2-site ELISA	fmol/ml	1.9	2.0	1.6 2.4	L	>91	79	5	19	24	0.0204
						H	39	46	7	6	13	
Big-ET-1 (22-38)	RIA	pg/ml	---	---	---	L	---	---	---	---	---	0.1441
						H	---	---	---	---	---	
ET-1	In-house RIA	pg/ml	---	---	---	L	---	---	---	---	---	0.2620
						H	---	---	---	---	---	

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

Figure 17. Patients with mild to moderate CHF (NYHA Class I-II)
A : N-proANP (1-98) < 3292 fmol/ml
B : N-proANP (1-98) > 3292 fmol/ml
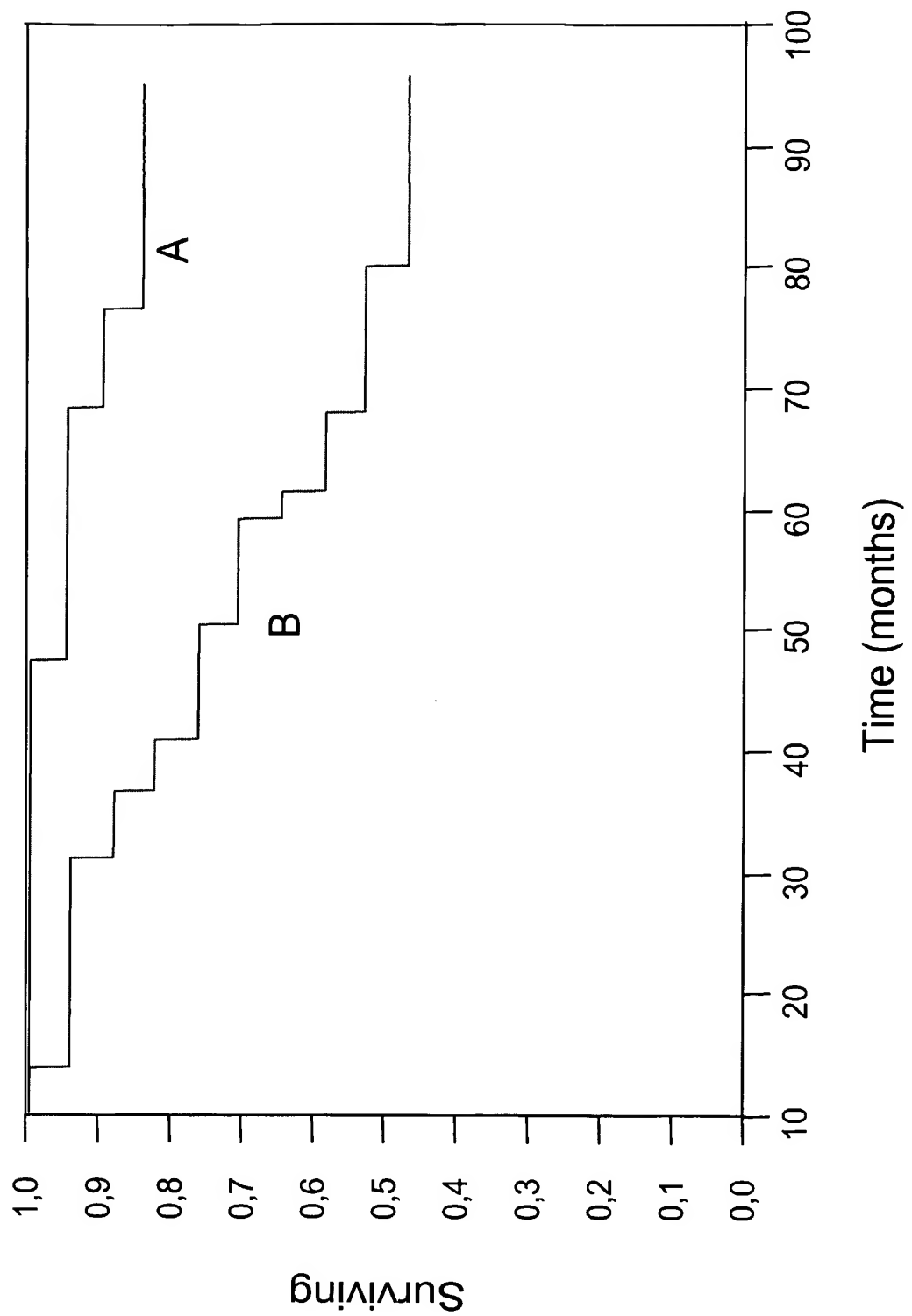
Figure 18. Patients with mild to moderate CHF (NYHA Class I-II)
A : N-proBNP < 404 fmol/ml
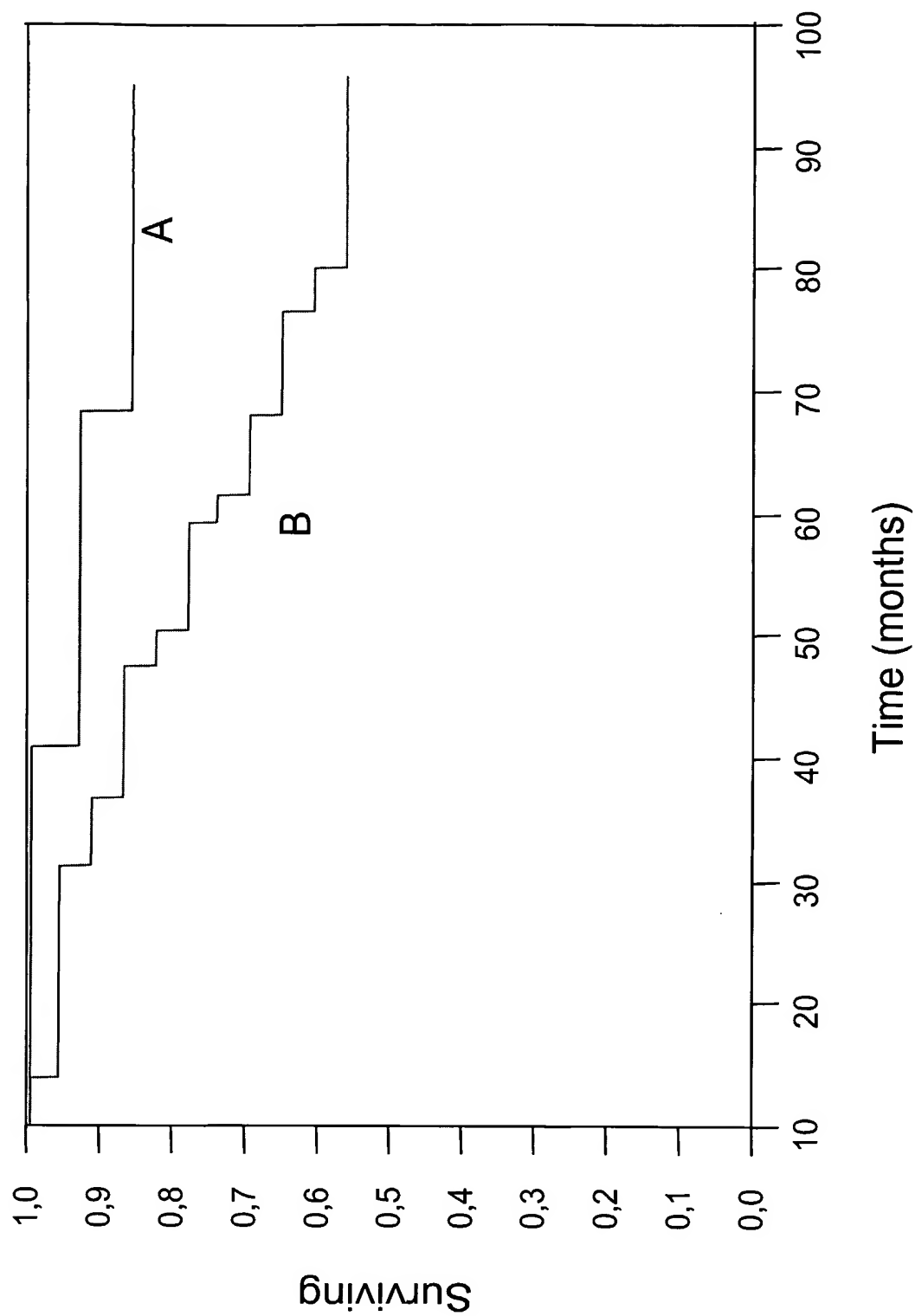B : N-proBNP > 404 fmol/ml

Figure 19. Patients with mild to moderate CHF (NYHA Class I-II)
A : BNP (RIA) < 25 pg/ml
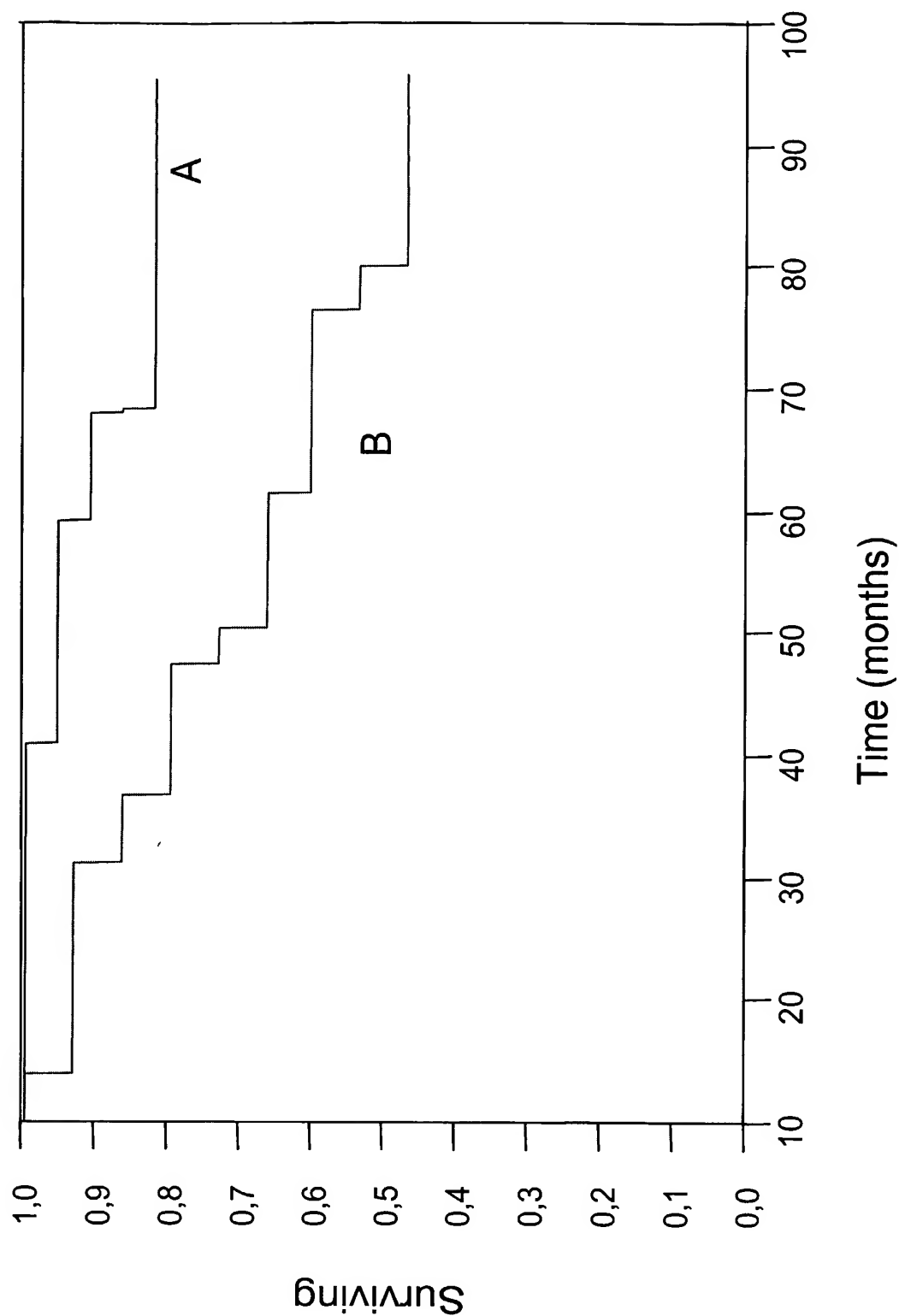B : BNP (RIA) > 25 pg/ml

Figure 20. Prognostic values for survival using N-proANP (1-98) testing determined with a specific 2-site ELISA, used in combination with several other natriuretic and vasoactive peptide assays in NYHA I-II patients *

Parameters considered in combination		Classification based on cut-off combinations (Cut-off levels are as in Fig 5) L : below cut-off H : above cut-off	% Survival at end of study	75% survival estimation (months)	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)								
N-proANP (1-98) (2-site ELISA)	BNP (IRMA)	M1 L and M2 L	92	>91	1	11	12		
		M1 H and M2 L OR M1 L and M2 H	60	70	4	6	10	36	0.0596
		M1 H and M2 H	50	42	7	7	14		
N-proANP (1-98) (2-site ELISA)	BNP (RIA)	M1 L and M2 L	93	>91	1	13	14		
		M1 H and M2 L OR M1 L and M2 H	58	59	5	7	12	36	0.0114
		M1 H and M2 H	40	35	6	4	10		

Parameters considered in combination		Classification based on cut-off combinations (Cut-off levels are as in Fig 5) L : below cut-off H : above cut-off	% Survival at end of study	75% survival estimation (months)	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)								
N-proANP (1-98) (2-site ELISA)	N-proBNP Competition one site ELISA	M1 L and M2 L	92	>91	1	11	12		
		M1 H and M2 L OR M1 L and M2 H	67	50	3	6	9	36	0.0417
		M1 H and M2 H	47	40	8	7	15		
N-proANP (1-98) (2-site ELISA)	Big ET-1 (2-site ELISA)	M1 L and M2 L	84	>91	3	16	19		
		M1 H and M2 L OR M1 L and M2 H	50	41	2	2	4	36	0.0365
		M1 H and M2 H	46	40	7	6	13		

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

Figure 21. Patients with mild to moderate CHF(NYHA Class I-II)
A : N-proANP (1-98) < 3292 fmol/ml AND N-proBNP < 404 fmol/ml
B : N-proANP (1-98) > 3292 fmol/ml OR N-proBNP > 404 fmol/ml
C: N-proANP (1-98) > 3292 fmol/ml AND N-proBNP >404 fmol/ml
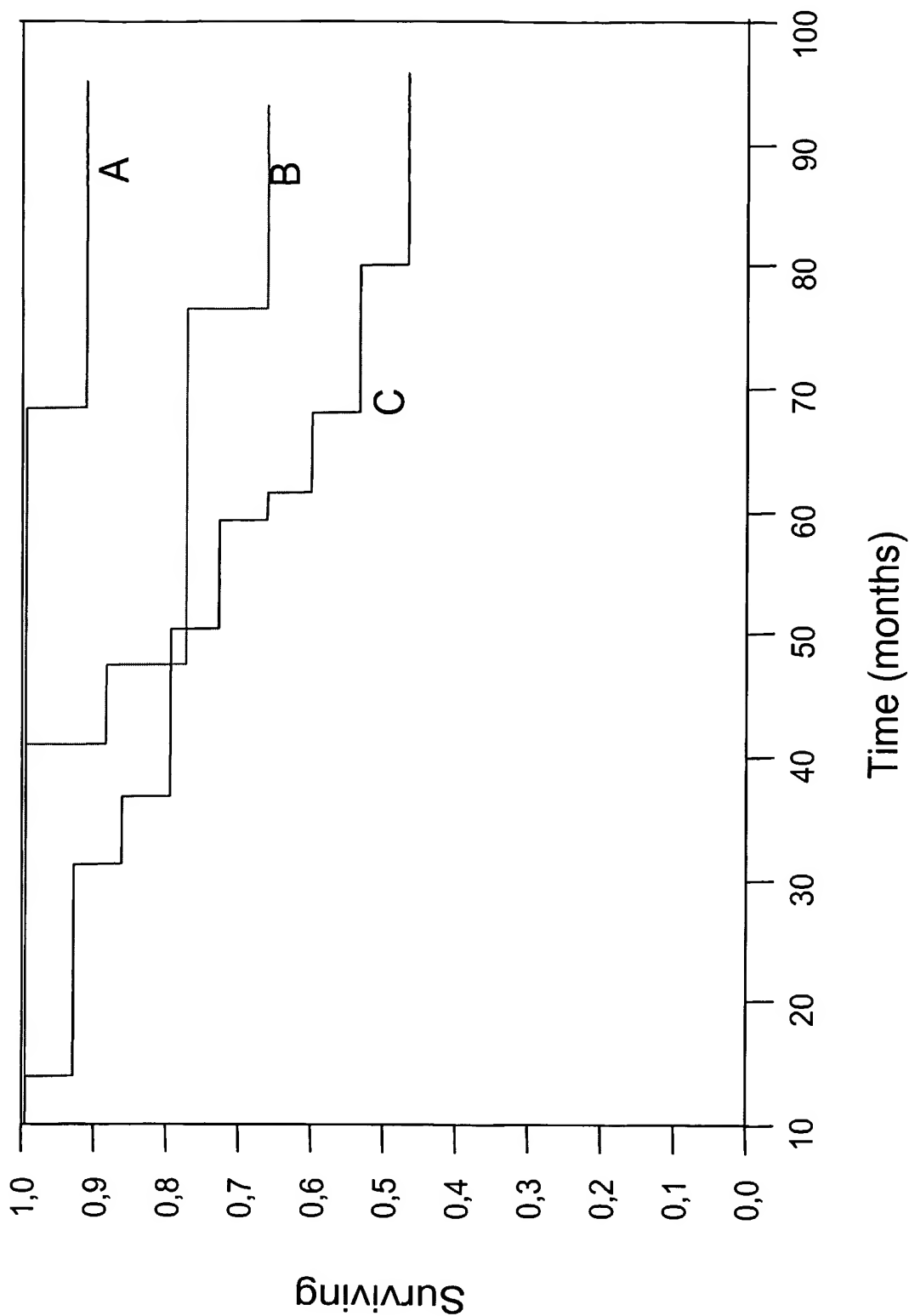
Figure 22. Patients with mild to moderate CHF (NYHA Class I-II)
A : N-proANP (1-98) <3292 fmol/ml AND BNP (RIA) <25 pg/ml
B : N-proANP (1-98) >3292 fmol/ml OR BNP (RIA) >25 pg/ml
C : N-proANP (1-98) >3292 fmol/ml AND BNP (RIA) >25 pg/ml